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EFFECT ON CHESTNUTS OF SUBSTANCES INJECTED INTO THEIR TRUNKS

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These observations are based on tree injections made during the summers of 1912, 1913, 1914, and 1915.[1] All the trees were orchard chestnuts: Paragon scions grafted on *Castanea dentata*.

TREES INJECTED WITH DYES

Some evidence concerning the path of the injected solution in the tree was obtained from stains. In 1912, 0.01 percent solutions of eosin, methyl green, and Congo red were injected into the trees. Although the holes were cut into the heartwood, the stains were found in the vessels of the youngest, or last annual ring; from here the stain spread slightly through the surrounding tissue. It was found that the stain descended as well as ascended in these vessels. In the roots the path of the stain was not followed to the root tips; it was found in the ring of vessels. In the small branches and twigs of the tree the stain often encircled the wood, whereas in the trunk lower down it had been seen in patches only in the last year's ring of vessels. The dyes varied in their effect on the trees. The eosin stain passed into the leaves of the injected trees and was toxic in the dilution used. Methyl green was not a good stain in that the color showed a tendency to fade. Illustrations of sections of these injected trees are in the report of the physiologist of the Pennsylvania Chestnut Blight Commission for the year 1912.[2]

In 1913, the non-toxic dyes, methylene blue, Congo red, and trypan blue, in 1/40 percent solution were injected for twenty days into six small trees, the injections starting April 16. At first the solutions flowed rapidly into the trees, especially the methylene blue, then more slowly, and had practically ceased going in when the injections were stopped. The trees were cut down in October. Two of these trees were infected with the

[1] Rumbold, C. The injection of chemicals into chestnut trees. Amer. Journ. Bot. 7: 1–20. 1920.

[2] Rumbold, C. Report of the physiologist. Report of the Pennsylvania Chestnut Tree Blight Commission, Harrisburg, July 1 to December 31, 1912, pp. 45–47, figs. 43–47. 1913.

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chestnut blight fungus; one had just been girdled at the base of the tree. The stain extended into the branches and roots, sometimes but part way, sometimes to the beginning of the year's growth. In no case did it extend beyond the beginning of the new year's growth of twigs and roots. At the point of injection it had penetrated three annual rings of wood and was found in the bark. However it was soon confined to the last annual ring in the neighborhood of the vessels. The girdled tree showed the stain passing through the diseased area by way of the vessels and tracheids; traces of it were found in the bark where disintegration had just begun. It will be noticed that the results of injection in 1912 and 1913 differed. No experiments were undertaken to discover the cause of this difference.

As stated before, most of the dye passed through the xylem elements of the last formed annual ring. At first it entered the large spring vessels, which appeared, however, not always to be the carriers. The summer tracheae or vessels, which in cross sections of Paragon chestnut form characteristic flame-like lines of pores diverging from the spring vessels, were deeply stained, and so were the tracheids. The trees injected with methylene blue showed ragged tissues and holes in the neighborhood of the spring vessels. There were traces of dye found in some of the vessels of the new growth of wood near the point of injection, but generally this new growth was unstained.

A microscopic examination of the xylem cells showed that the dye was retained by the walls of the vessels and tracheids through which it passed.

These phenomena (the decreasing size of the injected area and the gradual dilution of the solution as the distance from the point of injection increased) were observed when solutions of salts were injected into growing trees. Though the paths could not be followed as easily as when dyes were used, they could be traced, often, by a formation of abnormal bark tissue which disappeared as the distance from the point of injection increased. When a "killing" solution was injected the path was marked on the trunk by vertical strips of dead tissues. Those twigs and branches whose vascular system entered this path were killed; often but one side of a branch was affected. All stages of reaction to an injection could be seen in a tree: dead tissue at the point of injection in the trunk, dead or falling leaves on the branches nearest the injection hole, spotted leaves on branches higher up the tree, and no signs of injection visible in the top of the tree.

Time of Injection and Dilution of Solution

The way in which a tree was affected depended both on the time of the injection and on the dilution of the chemical solution. Concentrated solutions acted more quickly than dilute ones and generally were injurious.[3]

[3] "Experiments on the P_H or true acidity values of normal and cankered chestnut bark adjacent to the cambiam layer show that healthy chestnut bark has a P_H of about 4.8,

Alkali Metals

In September the effects of an injection of lithium carbonate 1/20 G.M. were visible in the leaves three days after the injection started. The leaves nearest the point of injection were first affected, those furthest distant last. When the dilution was 1/200 G.M. or weaker, the leaves first affected were at the ends of the branches; in August they were those nearest the burs. Trees injected in late summer with a 1/20 G.M. dilution produced normal leaves in the following spring at the normal time, but after the leaves were full grown they gradually showed the characteristic lithium curling and spotting (Pl. I, fig. A), although no more injections had been made in the trees. The other lithium salts acted in the same manner.

What happened in the case of the lithium salts, happened to some extent with the other alkali metals (ammonium compounds excepted). Sodium solutions 1/20 G.M. in strength produced effects like those of the lithium salts; but when diluted to 1/200 G.M. they did not blotch the leaves or affect the young bark, so that the presence of unusual quantities of sodium in the branches could not be vouched for. Potassium salts behaved like the sodium. It was observed in October that the sodium-, potassium-, and ammonium-injected trees (1/100 G.M. and 1/200 G.M.) generally lost the leaves on the ends of the branches near the burs first, and that this leaf fall was previous to the fall on the water-injected trees. The ammonium salts: chloride 1/200 G.M., carbonate 1/100 G.M., and hydroxide 1/100 G.M., also appeared to affect the leaves on the ends of the branches, causing them to drop; the sulphate 1/200 and 1/500 G.M. blotched the leaves. The normal growth of the trees was not seriously affected by the injection of the alkali metals.

Heavy Metals

Of the heavy metals injected, potassium chromate and bichromate and copper sulphate and chloride showed their effects most quickly. The chromates were more toxic and spread through the tree more quickly than the copper salts; the bichromate was more poisonous than the chromate.

The leaves of the tree injected with the chromate solutions became affected in 48 hours. The veins of the leaves browned first, then the leaves curled upward, dried, and dropped off. New leaves formed, but they in turn fell. The dilution 1/10000 G.M. of potassium chromate behaved like the 1/20 G.M. copper salts. With the exception of those trees injected with this latter dilution, the trees of this series were almost bare in August.

whereas samples of cankered bark have shown P_H values as low as 3.24. A P_H of 3.24 represents an acidity about 23.7 times that of 4.8. When N/100 and N/1000 alkalies are injected into the tree the acidity automatically increases very slightly as a kind of immunity to offset the effect of the alkali. When larger quantities of N/10 alkali are injected the sap becomes decidedly alkaline and the tree dies. The details of these investigations will appear later in the Journal of Agricultural Research." (C. Rumbold, M. R. Meacham and S. F. Acree.)

Ten days after being treated with potassium bichromate 1/1000 G.M., the back of the tree cracked along the edges of the path of the solution. In July and August, *Penicillium* sp. grew luxuriantly in these cracks and in the points of injection. The following year all the trees injected with the chromate solutions were dead.

The day after an injection of copper sulphate 1/20 G.M. was started, the leaves began to turn brown, those nearest the point of injection first. Copper chloride 1/20 G.M., zinc chloride 1/20 G.M., and barium chloride 1/20 G.M. acted almost as quickly. All of these were "killing" solutions. As previously stated, the paths these solutions took up and down the tree could be followed by the visible killing of the tissues. The region they passed through was a narrow one, but little wider than the hole made for the injection. Those twigs and branches whose fibers entered this path showed dead leaves. The leaves were the first to show the effects those nearest the point of injection browning first. A smell of decaying plant tissue became noticeable (in the case of Cu_2SO_4 1/20 G.M. in 10 hours, during which time 1 liter had been injected), which sometimes continued for one and two days. The dying leaves did not become crisp until some time after they had browned, in one case not until four days after browning. Environmental conditions probably influenced this phenomenon. Eventually all the leaves on the trees died, and soon those on the parts of the trees not included in the paths of the solutions fell off. The leaves dropped as they would in the autumn. The denuded branches quickly produced new leaves, so that trees injected in August had full-sized green leaves in December. The dead leaves still hung on the injected branches, rendering them conspicuous. The following spring these trees leafed, and produced fruit like the surrounding trees. The branches which had been injected were dead. The effect on the other parts was as though the trees had been severely pruned.

Colloidal Metals

The solutions of the heavy metals proved detrimental to the normal growth of the trees. The colloidal metallic solutions were exceptions. Examination of the injected trees indicated that most of the injected colloids stayed in the trunks near the place of injection.

Carbon Compounds

Two of the carbon compound solutions proved very toxic when injected. Four-tenths percent formaldehyde[4] affected the trees much as did the stronger concentrations of the copper solutions, but more severely for the reason that formaldehyde made broader paths when passing up the trunks. The trees above the point of injection were dead the following spring, but produced suckers from the base of the tree and from buds near the base.

[4] Schering.

Meta-cresol 1/1000 G.M. killed the tissues as it passed up and down the tree. The midribs and veins of the leaves browned and exuded a smell of creosote. Finally they turned black and shriveled, hanging to the twigs as though scorched by fire. Along the sides of the path of the solution callus formed. The bark peeled from the injected area and exposed the wood. Outside this path the tree was unaffected (Pl. I, figs. *C* and *D*).

The dilutions of the carbon compounds injected, with the two above noted exceptions, did not, apparently, seriously affect the normal growth of the trees, though some of them caused blotching of the leaves.

Extracts

Canker extract killed the trees. Water extract of healthy bark did not affect them.

Water

Water injected into trees for three succeeding years apparently in no way modified their growth.

Discoloration of Leaves Due to Injection

Some of the solutions injected affected the leaves in so marked a manner that one could tell from the type of blotching what base had been introduced.

Lithium produced the most characteristic blotches of all the substances. These blotches appeared irrespective of whether a carbonate, hydroxide, chloride, nitrate, or sulphate was introduced. Usually the tip and the edge of the leaf between the veins turned a reddish brown color, giving the leaf a scalloped appearance (Pl. III, fig. *A*). Sometimes, however, these spots appeared in the parenchyma in the middle of the leaf. A dark line separated the green from the brown area. The leaf curled upward. As more lithium accumulated, the discolored area advanced toward the midrib. The base of the leaf was the last to turn brown.

Sodium carbonate 1/20 G.M. killed the leaf parenchyma in somewhat large irregular areas, which sometimes were in the central part of the leaf extending across veins and leaving the leaf edges green. The division between green and brown areas was sharply defined. Dilute solutions of sodium salts did not blotch the leaves. The potassium salts in the dilutions used in the injections did not blotch the leaves.

Ammonium compounds did not brown the leaves, but ammonium sulphate 1/200 G. M. and 1/500 G.M. caused a wrinkling or frilling of the leaf edges. This frilled area became translucent and later brittle, and the network of small veins showed prominently. Occasionally these wrinkled areas looked bleached, and were surrounded by a dark green band.

The colloidal metals did not visibly affect the leaves.

Concentrated heavy metal solutions produced three varieties of discolored leaves; one, a browning of the midrib and veins, which gave the

leaves a finely checked appearance. The parenchyma browned last. The leaves then became dry and curled upward. There was another kind of discoloration characteristic of these solutions which appeared on leaves distant from the point of injection, or at a point where the solutions injected were diluted. Irregular brown spots appeared on the edges of the leaves which spread gradually toward the green petiole. The line of demarcation between brown and green areas was sharply defined. Such leaves were found on all trees injected with heavy metals. This effect in turn was quite different from that produced on leaves in the uninjected parts of trees treated with concentrated solutions, where a gradual bleaching appeared.

The manner in which formaldehyde 4/10 percent and meta-cresol 1/1000 G.M. affected leaves has been described. While meta-cresol proved so toxic, para-cresol 1/1000 G.M. produced no apparent effect on the leaves.

Those carbon-compound-injected trees which had discolored leaves showed two variations of discoloration. Para-nitro-phenol 1/500 G.M. browned the midribs and veins of leaves near the point of injection. Those leaves gathered from more distant parts showed light brown blotches on the edges which gradually advanced toward the base of the leaf. (The leaves, as far as appearance was concerned, could have been taken from a tree injected with $HgCl_2$ 1/1000 G.M.) Trees injected with the 1/1000 G.M. solution of para-nitro-phenol also showed these two varieties of discolored leaves. Ortho-nitro-phenol 1/1000 G.M. produced effects on leaves resembling those on ammonium-sulphate-injected trees, the leaves having translucent, brittle, frilled edges.

Picric acid 1/1000 G.M. caused the appearance of blotched and frilled leaves; citric acid 1/50 G.M., of blotched leaves; citric acid 1/500 G.M., of blotched and frilled leaves; acetic acid 1/500 G.M., of blotched leaves; formic acid 1/1000 G.M., of blotched leaves; salicylic acid 1/5000 G.M., of blotched and frilled leaves; pyrogallic acid 1/1000 G.M., of blotched leaves, the entire leaf finally turning a bright yellow and dropping off, as well as of frilled leaves; phloroglucine 1/1000 G.M., of frilled leaves; pyrocatechin 1/1000 G.M., of frilled leaves.

A possible explanation for these three variations in the discoloration of the leaves on an injected tree is that the leaves became impregnated in the course of the solution's spread with varying dilutions of the injected substance, those at a distance being impregnated with a much more dilute solution than those near the place of injection. The more concentrated solutions killed the tissues as they passed, thus browning the midribs and veins of the leaves, leaving the parenchyma green. When sufficiently dilute they flowed into the leaves without apparent harm, but gradually accumulated through transpiration in the parenchyma cells until a poisonous effect was produced. The third variation, that in which the leaf edges wrinkled or frilled, may be the effect not of the substance originally injected,

but of by-products resulting from injuries caused by its presence in the lower parts of the tree. These three variations did not appear on every injected tree; sometimes there was but one kind, sometimes there were but two kinds of discolored leaves.

Effect on Trunks

The holes made for the injections usually were filled with grafting wax after the removal of the injection tubes. A callus growing from both sides of the wound gradually closed it, leaving a small slit hardly noticeable on the tree. Sometimes this callus forced the wax from the hole, sometimes completely closed it in. It was found on examining felled trees that callus might cover the injection wound while an air space extended from the point of injection up and down the tree trunk between the outer bark and the wood (Pl. III, fig. *B*). This hole or tunnel was caused by the failure of the new annual ring to grow at that point, the cambium.layer having been killed by the injected fluid. Such holes, first noticed in trees injected with lithium salts, were found to be a somewhat usual result of injection. Trees treated with meta-cresol 1/1000 G.M., formaldehyde 0.4 percent, potassium bichromate 1/1000 G.M., or mercuric chloride 1/1000 G.M., showed these holes in marked degree in that the bark cracked and peeled away from the treated area. The lithium-injected tree, first noticed, had been injected in the late fall and the injection wound had been left uncovered. In the spring of the following year, this hole was found filled with water below the point of injection. It was uncovered by cutting away the bark. There was no chestnut blight infection found and callus had formed along its sides. It extended from a point at the base of the tree about three feet below the point of injection to a point somewhat less than three feet above the hole. It was thought that some of these holes might be formed beneath the bark by the eroding effect of the extraordinary amount of foreign fluid passing through a narrow channel rather than by the toxic character of the fluid. Trees injected with methylene blue showed this disintegration in a less marked form. Primarily, the nature of the solution injected determined the formation of these holes and their size, for an examination of the injected trees showed that weak acids, water, and extracts did not produce such holes. A tree into which para-nitro-phenol 1/1000 G.M. had been injected, and in which one injection ran for more than five weeks, showed short and rather narrow holes. The colloidal metals produced no holes, nor was there an abnormal growth of tissue.

The "killing" solutions produced no stimulation of growth further than the callus which cut off the dead tissue from the living. Solutions more dilute did not kill the tissue outright, but caused the formation of wound tissue in the growing annual ring and bark.

Effect on Fruits

All the injected trees with the exception of those treated with the concentrated solutions of the heavy metals and formaldehyde produced a normal appearing crop of nuts.

There was no sign of a stimulation of the trees by the substances injected further than that the nuts growing on trees treated with the alkali metals in general appeared somewhat larger and glossier than those on trees injected with water or carbon compounds. Lithium was found in the nuts gathered from the trees injected with the lithium salts. The contents of the nuts gathered from the other injected trees were not tested further than by a superficial feeding experiment with white rats[5] to test their possible poisonous effect. In view of the fact that lithium was found in the nuts, it seemed possible that some of the other injected substances had found their way into the fruits. The amount of poison in them must have been extremely small since they did not appear to injure the rats' health. Another indication of this lack of toxicity was a count made of the wormy nuts gathered from treated and untreated trees in the orchard. This count showed the percentage of wormy nuts to be the same for both classes of trees.

It seems possible, judging from the varying results of the injections made in the spring and fall, that the amount of injected substance which finally reaches the nuts can be influenced by the time of injection. The late summer injections quickly affected the chestnut fruits, as shown by the spotting of the burs and neighboring leaves when injected with lithium.

For the sake of brevity the substances injected are arranged as carefully as possible in groups according to the effect they produced on the trees during the summers of experimentation. Very often the trees did not respond in the same degree to injections of the same chemical so that it was difficult to judge its general effect, and possibly some of these dilutions of chemicals could be put in two groups.

No apparent effect on trees

Water
Water extract of healthy chestnut tree bark
Congo red 1/40 percent
Trypan blue 1/40 percent
Colloidal cuprous hydroxide 1/3300 G.M.
Colloidal metallic silver 1/6400 G.M.
Methyl alcohol 1/100 G.M.
Acetic acid 1/3000 G.M.
Formic acid 1/6000 G.M.
Lactic acid 1/1000, 1/2000 G.M.
Anilin sulphate 1/1000 G.M.
Sodium carbolate 1/1000 G.M.
Phenol Sodique, 1 cc. to 1000 cc. H_2O
Para-nitro-phenol 1/10000 G.M.
Para-cresol 1/1000 G.M.
Thymol 1/3000 G.M.
Oil of bitter almonds 1/10000 G.M.

[5] Chestnuts were gathered from each injected tree and kept separate in labeled paper bags. Twelve rats were fed regularly with the chestnuts. A day of chestnut feeding (the nuts for the day being those gathered from trees injected with a particular chemical) alternated with one of bread, milk, and grain.

The ammonium compounds 1/500 G.M.

Apparently a slight stimulant

The weaker dilutions of the alkali metals
Para-nitro-phenol 1/1000 G.M.
Picric acid 1/10000 G.M.

Slightly detrimental (blotched leaves, death of cambium near the point of injection)

Para-nitro-phenol 1/500 G.M.
Ortho-nitro-phenol 1/1000 G.M.
Picric acid 1/500, 1/1000 G.M.
Pyrocatechin 1/1000 G.M.
Pyrogallic acid 1/1000, 1/500 G.M.
Phloroglucine 1/1000 G.M.
Benzoic acid 1/500 G.M.
Phenol 1/1000, 1/500 G.M.
Copper sulphate 1/100 G.M.
Lithium salts 1/100 G.M.
Ammonium compounds 1/100 G.M.
Sodium chloride 1/100 G.M.
Eosin 1/40 percent
Methylene blue 1/40 percent
Acetic acid 1/1000 G.M.
Formic acid 1/1000 G.M.
Citric acid 1/50, 1/500 G.M.

Detrimental (death of injected part of tree or of whole tree)

Copper sulphate 1/20 G.M.
Copper chloride 1/20 G.M.
Zinc carbonate 1/20 G.M.
Mercuric chloride 1/1000 G.M.
Potassium chromate 1/1000, 1/10000 G.M.
Potassium bichromate 1/1000, 1/10000 G.M.
Barium chloride 1/20 G.M.
Alkali metals 1/20 G.M. (NaCl 1/50 G.M.)
Formalin 0.4 percent
Acetic acid 1/100 G.M.
Formic acid 1/100 G.M.
Lactic acid 1/100 G.M.
Anilin sulphate 1/100 G.M.
Meta-cresol 1/1000 G.M.
Benzoic acid 1/500 G.M.
Salicylic acid 1/100 G.M.
Water extract of chestnut blight canker

Summary

For four years observations have been made on the effect of chemical solutions injected into the trunks of chestnut trees.

1. Usually it was found that the visible effect of a solution on a tree varied with the distance from the point of injection.

2. The effect varied with the dilution of the solution and the month in which the injection was made.

3. In general the effect of the injection of the alkali metals was not detrimental to the trees; injection of heavy metals was detrimental; colloidal metals were not detrimental; organic compounds were not detrimental; water extract of chestnut blight canker was detrimental, healthy bark extract was not.

4. Many of the bases produced characteristic discoloration of the leaves.

5. Lithium was found in the nuts gathered from lithium-injected trees. The nuts gathered from the remaining trees were not tested sufficiently to show positively whether or not they contained any of the injected chemicals.

The Effect of the Injected Chemicals on the Fungus *Endothia parasitica*

The results of the injections on the growth of the chestnut blight canker on the chestnut tree have been so uncertain and varied that, were it not for the fact that the work must stop for the present, no results would be mentioned.

It seems best to give a history of the results as they presented themselves.

The first indication of an effect from the injected chemicals on the fungus was in the summer of 1913. The trees which had been injected in 1912 had been inoculated with the chestnut blight fungus in the fall. The fungous growth from these inoculations on those trees injected with alkali metals had an abnormal appearance. However, the fungus continued to grow and eventually killed the trees. This abnormal appearance of the fungus together with the fact that the alkali-injected trees had, as a whole, a thrifty look led to the decision to put more emphasis upon the injection of the alkali metals.

In 1914, measurements were made of the cankers caused by the inoculations of 1913. These showed that the cankers on the control trees averaged the same size as those on the alkali-injected trees. The measurements of the cankers on the other injected trees gave confused results. As a whole the injected trees had larger cankers than the uninjected.

In 1915, a dead canker was noticed on a tree, no. 185 E, which had been injected with lithium hydroxide in April, May, and June, 1913, and in June and July, 1914. The dead canker was not noticed at first for the reason that dead bark covered the area (Pl. IV, fig. *A*). Not until this bark was removed (as one would remove the scab from a healed wound) was it noticed that a healthy callus had cut out the cankerous growth (fig. *B*). This same effect was noticed on a tree injected with sodium carbonate and on a thymol-injected tree. In 1916 these trees again became infected, and in 1917 the new chestnut blight cankers on them were growing at the normal rate.

In the meantime a better method of injecting the trees had been devised (Pl. IV, fig. *C*). Injections were made on forest trees as well as on small orchard trees.

In 1916 the injections were made with lithium and sodium salts only. The injections were made in three different regions. One set of trees was injected in April, May, and June, the second in June, July, and August, the third in August, September, and October. The results of these injections showed in 1917 that sodium salts were not as effective as lithium salts. The lithium injections made in April, May, and June seemed to have the greatest effect, in that the cankers were not growing vigorously and the trees had started to form a callus about the diseased areas. All the check trees were dead at the time of the inspection. Those injections made in August, September, and October appeared to have had the least effect. In no case

had an injection definitely stopped the growth of a canker. No further inspection has been given these trees.

Summary of Results

This and the preceding paper[6] constitute a report on an attempt made to answer by experimentation the following questions:

1. What substances can be injected into living chestnut trees?
2. When can they be injected?
3. Where does the injected material go?
4. What is the effect on the tree?
5. What is the effect on *Endothia parasitica* growing on the tree?

A compilation of the records of injections made in living chestnut trees during the growing seasons for five years showed:

1. That the trees possess a considerable capacity for absorbing solutions of substances. Solutions of organic compounds went into the trees more readily than solutions of inorganic compounds, the "true solutions" more readily than the colloidal. Injected solutions, with a very few exceptions, were absorbed more readily than injected water. In the dilutions used in these experiments, the more concentrated the solutions were, the more readily they were absorbed by the trees.

2. In southeastern Pennsylvania, June was the best month for injection in so far as rate of intake was concerned; then came July, May, August, September, October, and April. The rate of intake varied more in April, May, and June than in the summer and autumn months, but obviously was dependent upon the local weather conditions.

3. Examination of the trees showed that the injected solutions as a rule passed through the vessels of the youngest annual ring of wood up and down the tree trunk in a zone whose width was usually but little more than that of the injection hole. They passed into the branches and leaves, and in the case of the lithium salts into the nuts. They passed into the roots.

4. In general, the injection of the alkali metals was not detrimental to the trees; injection of heavy metals was detrimental; colloidal metals were not detrimental; organic compounds were not detrimental; water extract of chestnut blight canker was detrimental, healthy bark extract was not. The effect varied with the dilution of the solution and with the month in which the injection was made. Many of the bases produced characteristic discolorations of the leaves. Usually the visible effect of a solution upon a tree varied with the distance from the point of injection. The injections can cause the appearance of pathological xylem in the tree trunks.

5. This work is not completed and the results are inconclusive. Dilute

[6] Rumbold, C. The injection of chemicals into chestnut trees. Amer. Journ. Bot. 7: 1–20. 1920.

solutions of lithium salts injected in the spring months may have an effect on the chestnut blight fungus in that the growth of the cankers on the injected trees appeared to be checked somewhat and the trees showed a tendency to form a callus about the canker.

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EXPLANATION OF PLATES III AND IV

PLATE III

FIG. *A*. Tree no. 185E. An 8-year-old, grafted tree 4.85 m. high, 8 cm. in diameter. Injected April 15 to June 25, 1913, with 10 liters of lithium hydroxide 1/500 G.M. Leaf collected June 20, 1913. This tree produced many large nuts in the autumn. The shaded areas in the illustration indicate the brown portions of the leaf.

FIG. *B*. Tree no. 21C. A 16-year-old grafted tree 5.5 m. high, 1.1 cm. in diameter. Injected June 20 to October 16, 1913, with 26 liters of lithium carbonate 1/500 G.M. A diagrammatic drawing showing a cross section of portion of the trunk. *a*. Holes running up and down the trunk caused by the death of the cambium layer in the path of the injected alkali. *b*. The irregular year ring of wood formed during the injection period.

FIG. *C*. Tree no. 114E. A 9-year-old grafted tree, 46 m. high, 9 cm. in diameter. Injected May 9 to 15, 1913, with 14½ liters of meta-cresol 1/1000 G.M. The branch was cut August 15. Callus had formed along the edges of the paths of the solution. *a*. Diagrammatic drawing of cross-section of small branch. *b*. This year's ring of wood, normal in structure. *c*. The edge of the creosote stain. All tissue reached by the creosote was killed.

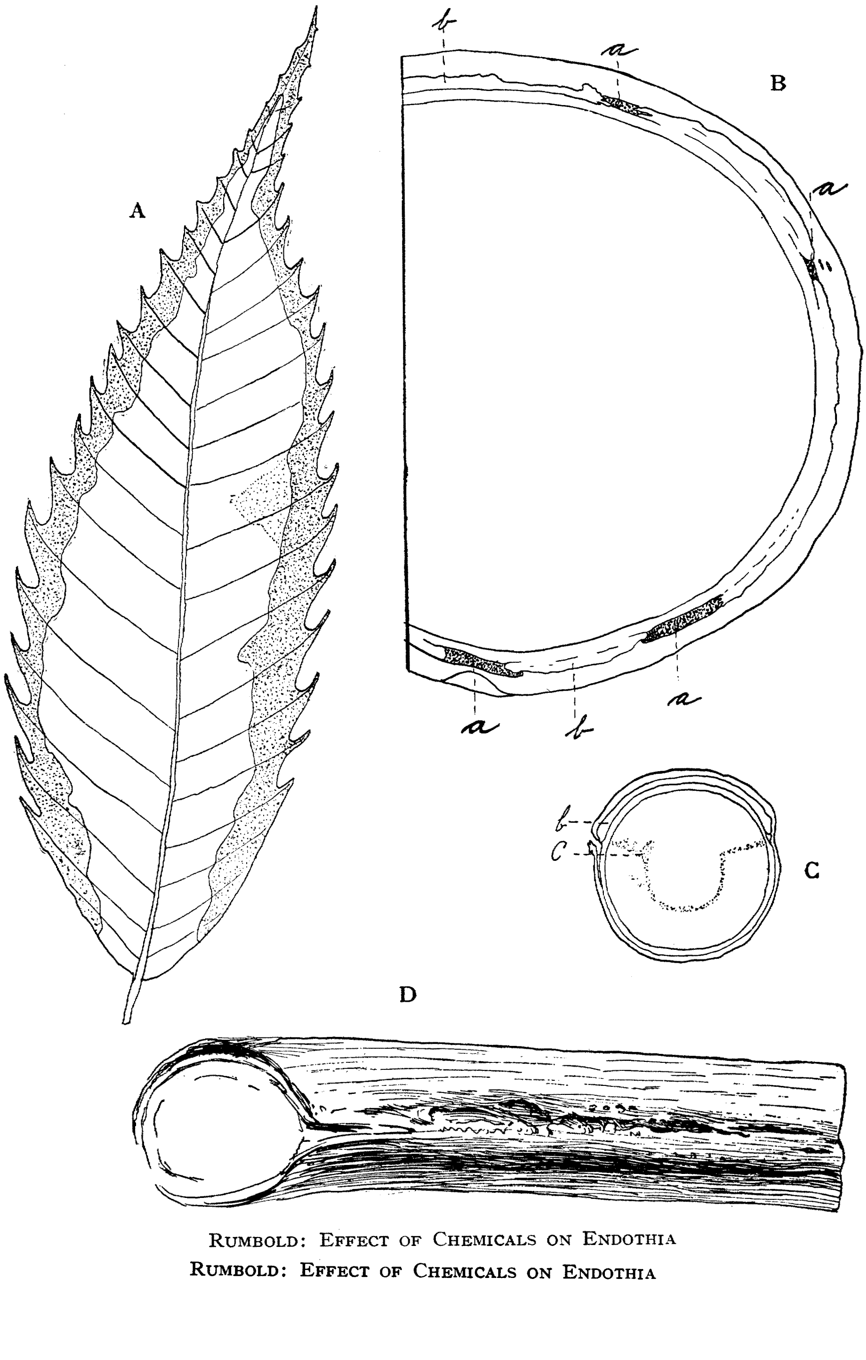
FIG. *D*. Tree no. 114E. Small branch showing an edge of a path of injected creosote solution. Normal callus separated the living bark tissue from the injected dead tissue.

PLATE IV

FIG. *A*. Tree no. 185E. An 8-year-old grafted tree, 4.85 m. high, 8 cm. in diameter. Injected April 15 to June 25, 1913, with 10 liters of LiOH 1/500 G.M. Injected again June 11 to June 17, 1914, with LiOH 1/200 G.M., and from June 26 to July 27 with 2 liters of LiOH 1/100 G.M. solution. Tree inoculated with *Endothia parasitica* October, 1913. Canker photographed October, 1915, when it was noticed that the canker had stopped growing.

FIG. *B*. Tree no. 185E. Same canker as above, photographed in November, 1915, when the dead bark formerly covering the canker had been pulled off. The clean, healthy callus which had "cut out" the fungus was thus disclosed. On the side branch can be seen the check made at the time the tree was inoculated. At the base of the photograph can be seen the upper part of a canker caused by a natural infection at the fork of a branch. This canker also had been "cut out" by a callus.

FIG. *C*. A method of injecting trees of any diameter. Link chains tightened by turnbuckles hold the rubber corks to the trees. Glass T-tubes thrust through the corks introduce the liquid into the injection holes. A tempered steel tube shaped like a cork-borer makes the hole for the injected solution. It can be driven into the tree through the horizontal arm of the T-tube after the apparatus is in place. A piece of rubber tubing is put on the free end of the horizontal arm of the tube, and the solution is cut off with a pinchcock after the drill is removed.



RUMBOLD: EFFECT OF CHEMICALS ON ENDOTHIA

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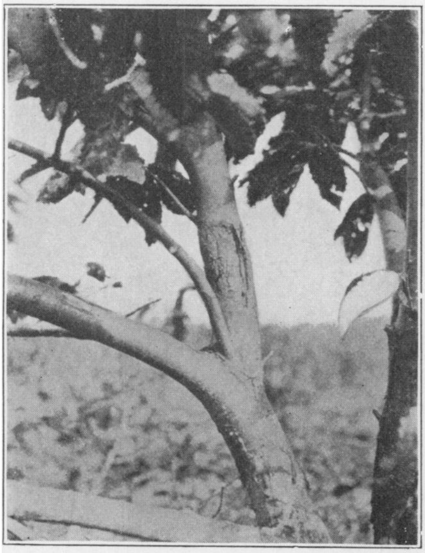
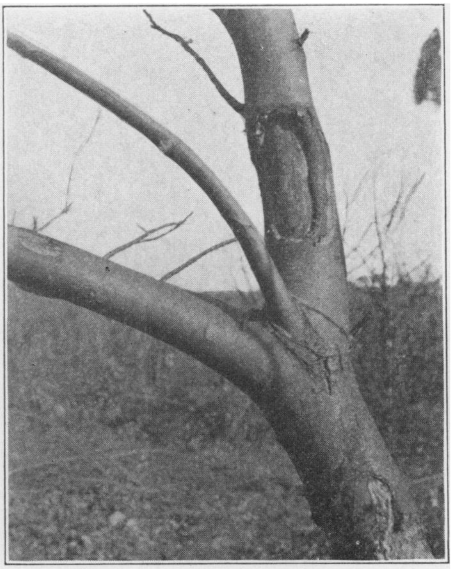


A



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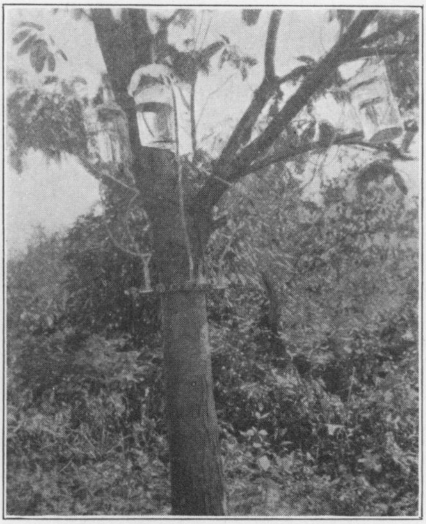

C

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